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Nucleotide sequence analysis and detection application of the coat protein gene of *Sweet potato latent virus* isolated from central Taiwan

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ABSTRACT

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A potyvirus-like agent (isolate CY) was isolated from sweet potato with leaf symptoms of chlorotic spots and vein mottling in Chia-Yi area, Taiwan. A 1.9-kb DNA product was amplified by reverse-transcription polymerase chain reaction (RT-PCR) from infected tissues of *C. quinoa* using the oligo(dT) and degenerate primers for potyviruses, cloned, and then sequenced. The cDNA fragment reflected 1880 nucleotides (nts) corresponding to the 3'-terminal region of a potyvirus and the sequence comparison indicated that the isolate CY belongs to *Sweet potato latent virus* (SPLV). The deduced amino acid sequence was determined as 561 residues that contains a part of the 3'-terminal region of NIb gene (268 residues) and the complete sequence of coat protein (CP) gene (293 residues). The 3'-terminal region of the cDNA was determined containing a non-coding region (NCR) of 197 nts. The DAG triplet for aphid transmissibility was found at the 7-9 residues from the N-terminus of CP gene. Multiple sequence alignment of the known sequences of SPLV indicated that the CP gene and the NCR of CY isolate share 96.5 and 100% nucleotide identities, respectively, with those of SPLV-TW. The phylogenetic analysis indicated that the CY isolate is closely related to Taiwan and China isolates, but distantly related to a Japan isolate. When a pair of specific primers designed from the CP gene of SPLV-CY was used for detection by RT-PCR, a 675 bp specific DNA fragment was amplified from SPLV-CY infected plants.

Key words: *Sweet potato latent virus*, gene cloning, sequence analysis, detection

INTRODUCTION

The occurrence of sweet potato virus diseases was first noticed in Taiwan in 1972 [10, 17]. Local lesion transfers and limiting dilution were used to separate individual viruses from mixed infections [3, 5, 8, 12]. Two different viruses were isolated from the plants of sweet potato [*Ipomoea batatas* (L.) Lam.] cultivar Tainung 63 showing interveinal chlorotic spotting [3, 9]. Both of them cause systemic symptoms on the plants of *I. nil* (L.) Roth [3, 9, 10] and their

natural hosts are restricted to the family *Convolvulaceae*. The difference in symptomatology, host range, aphid transmissibility and stability in saps distinguish the two viruses as *Sweet potato latent virus* (SPLV, originally designated as SPV-N) and *Sweet potato feathery mottle virus* (SPFMV, originally designated as SPV-A) [3, 9]. The two viruses are flexuous particles, approximately 700-750 nm in length, and induce typical cylindrical inclusions in the cytoplasm of infected cells [5, 12].

SPLV does not induce symptoms in most sweet potato varieties, but causes necrotic lesions on *Chenopodium quinoa* Willd. and systemic mosaic symptom on *Nicotiana benthamiana* L. Domin., while SPFMV dose not infect *N. benthamiana*, except the C1 strain [13]. Neither aphids nor whiteflies have been known to transmit SPLV, though it has some properties in common with potyviruses [6]. Since the isolate SPLV-TW (the isolate from Taiwan) was serologically related to potyviruses [11], it was assigned as a possible member of the genus *Potyvirus* pending sequence information [6].

The conventional taxonomy of the family *Potyviridae* is dependent on biological and serological relationships among its members. The recent increase in sequence data of the genus *Potyvirus* can be used as a more reliable taxonomic criterion of the members than those based on host range, symptomatology, or serological relationships [6]. Comparison of the core sequences of the potyviral coat protein (CP) genes clearly demonstrated a bimodal distribution of sequence identity among potyviral species and their strains, with the exception of some viruses which, though distinct, are more closely related to each other than to any other potyviruses [6].

In this study, we determined the nucleotide sequence of the CP and the 3' terminal part of the genome of a potyvirus-like agent (isolate CY) originated from a diseased sweet potato in Chia-Yi area, Taiwan. The nucleotide and the deduced amino acid sequences of the CP gene of the CY isolate were compared to those of potyviruses in GenBank and the isolate was identified as an isolate of SPLV. Based on the elucidated sequence, a specific primer pair was designed for detection of SPLV-CY infected plants.

MATERIALS AND METHODS

Plant materials and isolation of viruses

Sweet potato [(*I. batatas* (L.) Lam. cv. Tainung 66)] plants with interveinal chlorotic spotting at lower leaves were collected from Chia-Yi area as virus sources. Mechanical transmission was performed by grinding diseased tissues with a mortar and pestle in 0.1 M phosphate buffer, pH 7.5 [9], and the mixture was mechanically introduced, with cotton-tipped swabs onto carborundum-dusted leaves of *C. quinoa* plants. A pure virus culture was established after single-lesion transfers on plants of *C. quinoa*. Crude extracts of infected leaves of *C. quinoa* plants were negatively-stained with 2% dodeca phosphotungstic acid and examined by a JEOL-100 electron microscope (JEOL, Tokyo, Japan). The virus was maintained in this local lesion host that was used as the source for RNA extraction.

Viral RNA extraction and cDNA amplification

ULTRASPEC™ RNA isolation system (Biotex Laboratories, Houston, TX) was used to prepare total RNA from the *C. quinoa* plants showing necrotic lesions. Oligo(dT) primer was used for reverse transcription to synthesize the first strand cDNA from 2 μg of total RNA using the cDNA synthesis kit (Strategene, La Jolla, CA). From the reverse transcribed first strand cDNA, coat protein (CP) gene sequence was amplified, using EX-Taq polymerase (Takara, Shiga, Japan) according to the manufacturer's instructions and the forward (PotII, derived from conserved region of potyvirus the genomes [7]) and reverse oligo(dT) primers. The thermal cycling scheme used for 35 cycles of amplification was as follows: template denaturation at 94℃ for 1 min, primer annealing at 55℃ for 2 min, and DNA synthesis at 72℃ for 3 min. The amplification products were analyzed by electrophoresis on 1% agarose gel.

Cloning and sequencing

The PCR amplified products were cloned into PCRII-TOPO vector (TOPO TA cloning kit, Invitrogen, Carlsbad, CA), according to the instructions of the manufacturer. Plasmid clones with expected inserts were identified and used for sequencing [14]. Sequenceing of the cDNA clones was performed by dideoxynucleotide chain termination method [14, 15], using the T7 Sequenase version 2.0 DNA Sequenceing Kit (United States Biochemical Corp., Cleveland, OH) according to manufacturer's instructions. Nucleotide sequences were assembled and amino acid sequences were predicted using PC/GENE 6.85 software (IntelliGenetics, Inc., University of Geneva, Geneva, Switzerland). Sequence comparisons were performed with those from NCBI GenBank and EMBL databases. Multiple sequences were aligned by the PILEUP program in the BESTFIT program of the GCG package (Wisconsin Package version 10.0, Genetic computer Group, WI, USA). The phylogenetic tree was established with the deduced amino acid sequences of CP genes of SPLV isolates (Fig. 3) and potyviruses from the GenBank using

the NEIGHBOR-JOINING routines of PHYLIP software package version 3.63 (by Joseph Felsenstein and the University of Washington). The GenBank sequences (accession numbers in parenthesis) of potyvirus used for the analysis were listed here: BCMV (*Bean common mosaic virus*, NC 003397), BYMV (*Bean yellow mosaic virus*, D83749), CVbMV (*Chili vein-banding mottle virus*, U72193), DsMV (*Dasheen mosaic virus*, AF048981), LMV (*Lettuce mosaic virus*, X97705), OYMV (*Onion yellow dwarf virus*, NC005029), PepMoV (*Pepper mottle virus*, AB126033), PeSMV (*Peper severe mosaic virus*, AM1811350), PLDMV (*Papaya leaf distortion mosaic virus*, NC 005028), PPV (*Plum pox virus*, X81083), PRSV (*Papaya ringspot virus*, X97251), PSbMV (*Pea seedborne mosaic virus*, D10930), PVA (*Potato virus A*, Z21670), PVY (*Poato virus Y*, PVU09509), SMV (*Sugarcane mosaic virus*, NC 002634), SPFMV (*Sweet potato feathery mottle virus*, EF492048), SPLV (*Sweet potato latent virus*, EF492050), SPMSV (*Sweet potato mild specking virus*, SPU61228), SPVG (*Sweet potato virus* G, Z83314), SPVY (*Sweet potato virus* Y, AY4559611), TEV (*Tobacco etch virus*, M15239), TuMV (*Turnip mosaic virus*, AF530055), WMV2 (*Watermelon mosaic virus* 2, AB218280), YMV (*Yam mosaic virus*, YMU42596), and ZYMV (*Zucchini yellow mosaic virus*, NC003224). The repeatability of the branching orders obtained was estimated using the SEQBOOT program of (PHYLIP software) for bootstrap resampling (1000 bootstrap reiterations) the multiple sequence alignment. Bootstrap consensus trees were then built using the CONSENSE program and the NEIGHBOR unrooted tree was drawn using the DRAWTREE program of the PHYLIP software.

The specific primers designed for detection of SPLV-CY infected plants

According to the nucleotide sequence of the CP gene of SPLV-CY, primer L166 (5'-GACAGAGATATCAACACTGGCACC -3') and L841 (5'-TCCAAGTAGTGTGTGTATGTTCCG-3') were designed for detection of the virus in the infected plants by RT-PCR, which was performed as described above.

RESULTS

Isolation of the virus

After mechanical introduction of the extract from the diseased sweet potato (cv. Tainung 66) with interveinal chlorotic spotting (Fig. 1A), chlorotic spots developed on plants of *C. quinoa* 7 days post inoculation (dpi), which eventually turned into necrotic lesions 25 dpi (Fig. 1B). A single-lesion isolated virus induced systemic mosaic on plants of *N. benthamiana*. The host reactions were similar to those reported by of Liao *et al* [10]. When the crude extracts from leaves of *C. quinoa* were examined with JOEL electron microscope by leaf-dip method, virus particles of flexuous rods, 700-750 nm in length, were observed (data not shown). A typical isolate, designated as CY isolate, was selected for further studies.

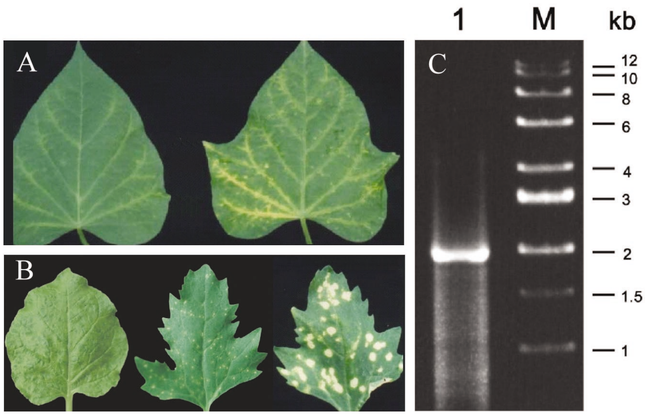

Fig. 1. (A) Symptoms on sweet potato cultivar TN66 with chlorotic spots and vein mottling collected from field. (B) Host reactions of the virus isolate CY isolated from the diseased sweet potato plant. Tobacco (*N. benthamiana*) showing mosaic symptom at 9 days post-inoculation (dpi) (left), chlorotic spots on *C. quinoa* at 7 dpi (middle), turning necrotic at 25 dpi (right). (C) Agarose gel electrophoresis of the DNA product amplified by reverse transcription-polymerase chain reaction (RT-PCR). A DNA fragment was amplified from total RNA isolated from infected tissue of *C. quinoa*, with primers oligo (dT) and PotII by RT-PCR. Lane 1, the 1.9 kb DNA product. Lane M, DNA standard markers (GeneMark, Taichung, Taiwan).

RT-PCR and cloning

The *C. quinoa* plants infected with CY isolate were maintained in the greenhouse as the source for total RNA extraction. A DNA fragment of about 1.9-kb was amplified by RT-PCR from the total RNA extracted from of CY-infected tissue by a potyvirus-specific primer PotII and oligo(dT) primer (Fig. 1C). The PCR product was cloned into PCRII-TOPO vector and subsequently sequenced. The 1880 bp DNA fragment reflected the 3'-terminal region of a potyviral genome, including a part of NIb protein gene (804 nts), complete CP gene (879 nts) and 3' non-coding region (3'-NCR) of 197 nts followed by a poly-A tail (Fig. 2). The deduced amino acid sequences of the partial NIb and the complete CP gene contained 268 and 293 residues, respectively. As with other potyviruses [16], the putative hydrolytic cleavage site VHHQ/A between

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5'-CAATTTGACAGTTCTCTTTCTCCTTACTTGATAAATGCAGTGTTACAATTACGCTTGCATTTTATGGAGGAATGGGACATAGGAGAGCAA   90
   Q  F  D  S  S  L  S  P  Y  L  I  N  A  V  L  Q  L  R  L  H  F  M  E  E  W  D  I  G  E  Q

   ATGCTGAGAAATCTGTATACTGAGATCGTCTACACACCAATACTGACACCAGACGGCACAATCATAAAGAAGCACAAAGGAAATAATAGC  180
   M  L  R  N  L  Y  T  E  I  V  Y  T  P  I  L  T  P  D  G  T  I  I  K  K  H  K  G  N  N  S

   GGACAACCATCAACAGTGGTTGATAATACATTGATGGTTATAATAGCAATGTATTATTCGCTACATGCAGAAGGTTATTCAATGAATGAA  270
   G  Q  P  S  T  V  V  D  N  T  L  M  V  I  I  A  M  Y  Y  S  L  H  A  E  G  Y  S  M  N  E

   GCCGAGTCAGTGTGTAAGTTCTTTGTAAACGGTGATGATTTGCTAATAGCTGTGCATCCTGAACATGAAGCTATATTGGACAAACTACAA  360
   A  E  S  V  C  K  F  F  V  N  G  D  D  L  L  I  A  V  H  P  E  H  E  A  I  L  D  K  L  Q

   TCTCATTTCAAGCAATTGGGTCTCAAGTACACATTTGATGATAGACATGACGACAAGTCGTCTCTTTGGTTCATGTCTCACCAAGGTATT  450
   S  H  F  K  Q  L  G  L  K  Y  T  F  D  D  R  H  D  D  K  S  S  L  W  F  M  S  H  Q  G  I

   AAACGCGATAACATGTACATTCCTAAGCTTGAAAAGGAACGGATAGTCTCAATCCTTGAGTGGGATAGAGCACGGGAACCAGTGCACAGG  540
   K  R  D  N  M  Y  I  P  K  L  E  K  E  R  I  V  S  I  L  E  W  D  R  A  R  E  P  V  H  R

   ATGGAGGCGATATGCGCTGCAATGGTTGAAGCATGGGGCTACGATGATTTGGTGCATGAAATTCGGAAATTTTATAAATGGTTGTTAGAT  630
   M  E  A  I  C  A  A  M  V  E  A  W  G  Y  D  D  L  V  H  E  I  R  K  F  Y  K  W  L  L  D

   CAGGCACCATACTCTGACTTAGCTAAGAATGGTGGAGCGCCATATATTGCAGAGGTAGCGCTGCGGAAATTATATACATCCGTAGCACCA  720
   Q  A  P  Y  S  D  L  A  K  N  G  G  A  P  Y  I  A  E  V  A  L  R  K  L  Y  T  S  V  A  P

   GACCGATCTGAGTTGGAGCGATATTTAGCAGCGATAATGGAGGAGCCAGAATTCGATGAAGAGGATGCGAATGTACACCACCAAGCCGAT  810
   D  R  S  E  L  E  R  Y  L  A  A  I  M  E  E  P  E  F  D  E  E  D  A  N  V  H  H  Q/ A  D

   GAAACCATTATCGATGCTGGAAAAATGGAAACGAAGAAGAAGCGGAACACAGAAGGCACACCACCACAGACTAACACACCAACCCAGCCA  900
   E  T  I  I [D  A  G] K  M  E  T  K  K  K  R  N  T  E  G  T  P  P  Q  T  N  T  P  T  Q  P

   AGTCCATCAGATAAGGGCAAGGAAGTTATATCAGCAAAAGGGGGAGAGATTACCCGCACATCAGAACATGACAGAGATATCAACACTGGC  990
   S  P  S  D  K  G  K  E  V  I  S  A  K  G  G  E  I  T  R  T  S  E  H  D  R  D  I  N  T  G

   ACCCTCGGAACTTTCCAGGTACCCCGCTTGCGTGCAATACCCACAAAAATTCGTCTGCCACTTGTTAAATCTGGAGCTGCATTGAACTTG 1080
   T  L  G  T  F  Q  V  P  R  L  R  A  I  P  T  K  I  R  L  P  L  V  K  S  G  A  A  L  N  L

   GATCATCTGTTAGTGTATAAGCCATCACAGCTAGACATAACAAATGCCAAGCCTACAAGGAGTCAGTTCAATCAATGGTATGAAGGCGTG 1170
   D  H  L  L  V  Y  K  P  S  Q  L  D  I  T  N  A  K  P  T  R  S  Q  F  N  Q  W  Y  E  G  V

   AAGAATGCTTACGAAGTGGATGACCAGCAGATGTCAATCTTGATGAATGGACTTGTTGTATGGTGTATAGAGAATGGGACATCTCCGAAT 1260
   K  N  A  Y  E  V  D  D  Q  Q  M  S  I  L  M  N  G  L  V  V  W  C  I  E  N  G  T  S  P  N

   ATCAATGGTGATTGGGTGATGATGGACGGAGATACACAAGTATCATACCCAATAAAGCCACTTATAGATTTCGCAGCACCAACATTTCGT 1350
   I  N  G  D  W  V  M  M  D  G  D  T  Q  V  S  Y  P  I  K  P  L  I  D  F  A  A  P  T  F  R

   CAAATAATGAAGCATTTTAGCGATGTCGCAGAAGCCTACATACAAATGCGCAATGCAGAGCAACCATACATGCCAAGATATGGTTTGCAA 1440
   Q  I  M  K  H  F  S  D  V  A  E  A  Y  I  Q  M  R  N  A  E  Q  P  Y  M  P  R  Y  G  L  Q

   CGGAACTTAACAGATATGTCACTGGCCCGATATGCTTTCGATTTTTACGAGGTGACGTCACGCACACCCATTCGTGCTAAGGAAGCGTAT 1530
   R  N  L  T  D  M  S  L  A  R  Y  A  F  D  F  Y  E  V  T  S  R  T  P  I  R  A  K  E  A  Y

   TTCCAGATGAAAGCTGCAGCGCTCACAAATACACATCATCGGCTGTTCGGTTTAGACGGAAATGTTTCAACCACTGAGGAAAACACCGAG 1620
   F  Q  M  K  A  A  A  L  T  N  T  H  H  R  L  F  G  L  D  G  N  V  S  T  T  E  E  N  T  E

   CGGCATACTGCAACAGATGTGGACCGGAACATACACACACTACTTGGAATGCGTGGCATTCATTAGTGTGCGTACTTATCTATAAAGTTA 1710
   R  H  T  A  T  D  V  D  R  N  I  H  T  L  L  G  M  R  G  I  H  *

   TTTATATTTATGTATGTATGTTCGTCGAAGGGGAATCTATTTGTGGAGTGTTGTGAGGGTATCCCTCGAGCAAAACATATAGTTCGATCC 1800

   TTTCTATTATATTTCAAGAATTTTATAACATTAGAGAGGCACTGCCTCCAGTCATAAACTTTTTGTCTTATACCAGAGAC-3'        1880
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Fig. 2. The nucleotide and deduced amino acid sequences of the cloned 3'-terminal region of the genome of the virus isolate CY collected from diseased sweet potato (Accession No. EF492050). The cloned 1880 nucleotides of the CY cDNA are shown starting from the first nt. The termination codon is indicated by an asterisk, followed by 197 nts of non-coding region (NCR). The putative protease cleavage site (Q/A) between nuclear inclusion protein b (NIb) and coat protein (CP), as well as the core of CP are underlined. The DAG triplet, a genetic indication for aphid transmissibility is boxed.

NIb and the CP was predicted for CY isolate (Fig. 2). The first significant domain of potyviral CP, the DAG box, involved in aphid transmission, was located at N-terminal 7-9 residues of the CP of CY. The potyviral 3'-NCR consensus sequences GAGG (nts 1032-1035) and CCTC (nts 1041-1044), separated by a 5 nucleotide intervening sequence, with the ability to form a stem-loop structure possibly having a role in replication [2, 6], were also found present in the sequence of CY isolate.

Sequence comparisons between isolates of SPLV and potyviruses

The nucleotide and deduced amino acid sequences of the CP gene of CY isolate were aligned with those of

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SPLV-JP   ..G.....C..T..CC.T........G..GG.A..T.....A.G...C..C..T....GC.AG...T...AG.AT.C..G....G..C.A..A..C..AG 100
SPLV-CH2  .....C........C...............G.A...G..........T.....................T........................A..... 100
SPLV-CH   .....C........C...............G.....G..........T.A.................A.....G.......................... 100
SPLV-TW   ..............CC.....A........G..................A.G..........A.................................G... 100
SPLV-CY   GCCGATGAAACCATTATCGATGCTGGAAAAATGGAAACGAAGAAGAAGCGGAACACAGAAGGCACACCACCACAGACTAACACACCAACCCAGCCAAGTC 100

SPLV-JP   TTGAG........T.....GA.AGCCC...TTGG...A.....AG..G..........C..G.T...T..G..C..A........T..T..T.....G.. 200
SPLV-CH2  ....G........T...............T.................G........C........................................... 200
SPLV-CH   ....G........T...............T.....A............T.......C.G......................................... 197
SPLV-TW   ......G......T.......................A.........................................T.................... 200
SPLV-CY   CATCAGATAAGGGCAAGGAAGTTATATCAGCAAAAGGGGGAGAGATTACCCGCACATCAGAACATGACAGAGATATCAACACTGGCACCCTCGGAACTTT 200

SPLV-JP   .........T..AC.......T.................CT.A.....A.....G........A..G.....TC.C......T.AC.G...........G 300
SPLV-CH2  ...A..............A................................C....................T.......................A... 300
SPLV-CH   ..................A................................C....G.................................A.....A... 297
SPLV-TW   ................................G................A......G........................................... 300
SPLV-CY   CCAGGTACCCCGCTTGCGTGCAATACCCACAAAAATTCGTCTGCCACTTGTTAAATCTGGAGCTGCATTGAACTTGGATCATCTGTTAGTGTATAAGCCA 300

SPLV-JP   ..G.....G..T..T..G.....T..AG.C..T..............C........C.....T...........A......................... 400
SPLV-CH2  ...........................G...............................G........................................ 400
SPLV-CH   ...........................G...............................G..........................C............. 397
SPLV-TW   ...........................G...........................................................C.T.......... 400
SPLV-CY   TCACAGCTAGACATAACAAATGCCAAGCCTACAAGGAGTCAGTTCAATCAATGGTATGAAGGCGTGAAGAATGCTTACGAAGTGGATGACCAGCAGATGT 400

SPLV-JP   .G....................A..G...........A.....A.....C..A...........C.................T.....C..G........ 500
SPLV-CH2  ..............................................T..C..A...................................C..G........ 500
SPLV-CH   ...................C....................C.....T.....A...................................C..G........ 497
SPLV-TW   ........................................C...........A....................A..............C........... 500
SPLV-CY   CAATCTTGATGAATGGACTTGTTGTATGGTGTATAGAGAATGGGACATCTCCGAATATCAATGGTGATTGGGTGATGATGGACGGAGATACACAAGTATC 500

SPLV-JP   ......G........C...........T..G...........CA.G..G...........C.....T....................C............ 600
SPLV-CH2  G.......................C..T..G...........C.....G...........C.......................T.....G......... 600
SPLV-CH   ........................C..T..G...........C.....G...........C...........T...........T.....G......... 597
SPLV-TW   .........................................................A..............T...........T............... 600
SPLV-CY   ATACCCAATAAAGCCACTTATAGATTTCGCAGCACCAACATTTCGTCAAATAATGAAGCATTTTAGCGATGTCGCAGAAGCCTACATACAAATGCGCAAT 600

SPLV-JP   .....A.....G...........G........A..G..A........G........TT....T..T..C..G..T.....C..T..A..T..A....... 700
SPLV-CH2  ....................G.............................C.....T.....T..T..............C........A..A....... 700
SPLV-CH   ..........................................C.T.....C.....T.....T..G..............C.....A..A..A....... 697
SPLV-TW   ..........................................C.T...........T.................T..............T..A....... 700
SPLV-CY   GCAGAGCAACCATACATGCCAAGATATGGTTTGCAACGGAACTTAACAGATATGTCACTGGCCCGATATGCTTTCGATTTTTACGAGGTGACGTCACGCA 700

SPLV-JP   .G.....C..G..C........A..C.....................................................G..T........C..T..... 800
SPLV-CH2  ..........C..C........A..C................T..................................C.G..T........C........ 800
SPLV-CH   ..........C..C........A..C...................................................C.G..T........C........ 797
SPLV-TW   ...................G..A..C........................................................T................. 800
SPLV-CY   CACCCATTCGTGCTAAGGAAGCGTATTTCCAGATGAAAGCTGCAGCGCTCACAAATACACATCATCGGCTGTTCGGTTTAGACGGAAATGTTTCAACCAC 800

SPLV-JP   ...A.................C........G............................................C...... 882
SPLV-CH2  ...........................................................................C...... 882
SPLV-CH   C..............A...........................................................C...... 879
SPLV-TW   .................................................................................. 882
SPLV-CY   TGAGGAAAACACCGAGCGGCATACTGCAACAGATGTGGACCGGAACATACACACACTACTTGGAATGCGTGGCATTCATTAG 882
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Fig. 3. Alignment of the nucleotide sequences of the coat protein gene of isolate CY with isolates of *Sweet potato latent virus* (SPLV) in GenBank, SPLV-CH (China, Goangzhou, X84011), SPLV-JP (Japan, E15420), SPLV-TW (Taiwan, X84012) and SPLV-CH2 (China, Henan, DQ399862).

SPLV isolates and selected potyviruses. Pairwise percent nucleotide and amino acid sequence identities among the complete CP genes of isolate CY and four other isolates of SPLV were analyzed. The highest identities, i.e., 96.5% nucleotide and 100% amino acid, were found with the isolate SPLV-TW (Taiwan isolate), followed by, 94.1% and 96.6% with SPLV-CH2 (China isolate), 93.4% and 96.4% with SPLV-CH (China isolate), and 82.4 % and 93.4% with SPLV-JP (Japan isolate). The result of phylogenetic analysis of the deduced amino acid of the CPs of SPLV isolates confirmed that CY isolate is an isolate of SPLV, with a closer relationship with the Taiwan and China isolates, but distantly related to the Japan isolate (Fig. 4).

The alignment of amino acids sequences of CP genes among the CY isolate and other previously reported

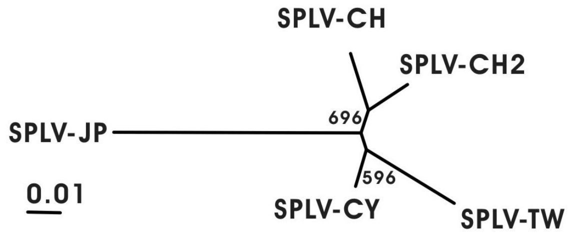

Fig. 4. Phylogenetic analysis of the coat proteins of SPLV isolates. The phylogenetic tree was established from the deduced amino acid sequences of the coat proteins of SPLV isolates from the GenBank using the NEIGHBOR-JOINING routines of PHYLIP software package version 3.63 (by Joseph Felsenstein and the University of Washington). The dendragraph produced using the NEIGHBOR-JOINING algorithm with 1000 bootstrap replicates is shown at appropriate nodes. The scale for genetic distances is indicated at the lower left.

potyvruses collected from the GenBank and EMBL databases was analyzed. A dendrogram of sequence relationship was generated from the alignment of the CP sequences. The phylogenetic tree indicated that SPLV was genetically closer to TEV, TuMV, and PVA, whereas it was distantly related to SPFMV, SPVG and SPVY (Fig. 5).

Detection of the infected plants by RT-PCR

When the specific primer L166 and L841 were used for detection of CY virus in infected plants, a 675 bp DNA fragment was amplified from the CY-infected plants of *C. quinoa*, *N. benthamiana* and varieties of sweet potato (Fig. 6). This amplified DNA fragment, in combination with the 1300 bp fragment amplified by potyvirus-specific primer pair PotI/PotII (Fig. 6), can be used to identify the infection by the CY virus.

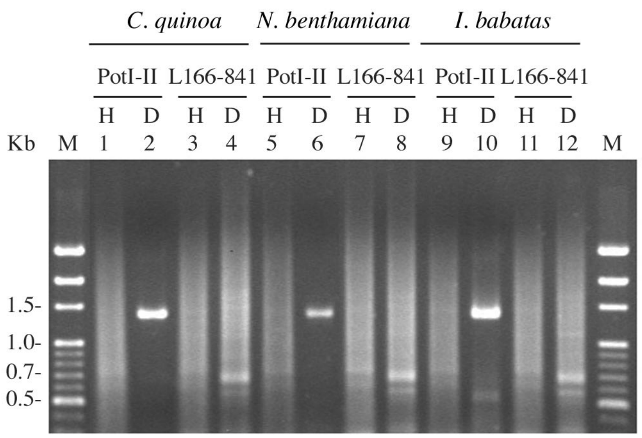

Fig. 6. Detection of the isolate CY from infected plants by reverse transcription-polymerase reaction, using the potyvirus-specific degenerate primers (PotI and PotII) and the specific primers (L166 and L841) designed from CY sequence. Lane M, DNA standard markers; Lanes 1-4, *C. quinoa* with necrotic lesions 7 dpi; Lanes 5-8, tobacco plants (*N. benthamiana*) with mosaic symptom; Lanes 9-12, plants of sweet potato cv. TN 57 with mottle symptom; H, uninfected controls; D, diseased plants.

DISCUSSION

A virus culture, designated as CY isolate, was originated from diseased sweet potato plants with symptoms of chlorotic spot and vein mottling in Chia-Yi area. The host reactions of CY isolate on sweet potato, *C. quinoa* and *N. benthamiana* were similar to those induced by the virus described as SPV-N in Taiwan previously [3, 9]. Electron microscopy also indicated that CY isolate is a potential member of potyviruses. Here we also presented molecular evidence of the CY isolate by the sequence analysis of its 3' genomic region, including the CP gene and the 3'-NCR. The isolate CY was found to have the highest nucleotide identity with an isolate of SPLV originated from Taiwan, followed by two China strains, and to be distantly related to a Japan strain. Thus, the isolate CY is identified as an isolate of SPLV, designated as SPLV-CY.

From the presence of the DAG box at the N-terminal region of CY CP, which is essential for aphid transmissibility of potyviruses [1], SPLV-CY is expected to be a typical aphid-transmissible potyvirus. The triplet is similar to that of SPLV-CH and SPLV-CH2 but different from SPLV-TW [6] that has a DTG instead. Our phylogenetic analyses also revealed that SPLV, TuMV, PVA and TEV are closely related potyviruses, reflecting a more recent evolutionary relationship of these viruses, whereas SPLV is distantly related to the sweet potato infecting viruses such as SPVY, SPVG and SPFMV, which may evolved from a different evolutionary pathway.

Sweet potato plants infected with SPLV normally do not show prominent symptoms under field conditions. But when co-infected with SPFMV, severe symptoms develop and the yield of sweet potato is tremendously decreased [4]. Therefore, to avoid the loss caused by SPLV, it is crucial to develop a reliable diagnosis tool for detecting the presence of the virus in the crop. This is especially important for the establishment of a SPLV-free stock for propagating a particular variety of sweet potato by tissue culture. The

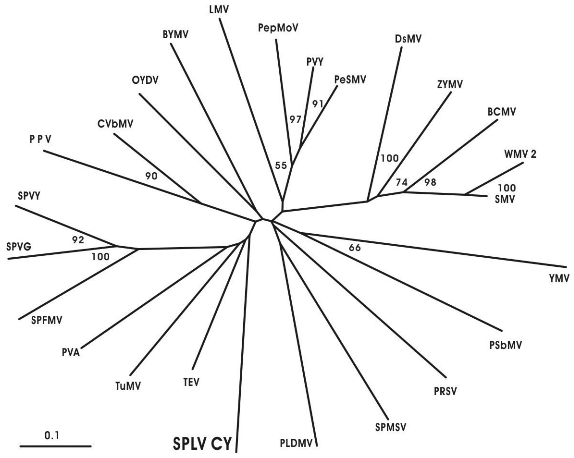

Fig. 5. A phylogenetic tree calculated from the deduced amino acid sequences of the coat proteins of 24 potyviruses with highest percent identities selected from GenBank, using the NEIGHBOR-JOINING routines of PHYLIP software package version 3.63 (by Joseph Felsenstein and the University of Wasington). The dendragraph produced using the NEIGHBOR-JOINING algorithm with 100 bootstrap replicates was shown at appropriate nodes. The scale to measure genetic distances is indicated at the lower left.

specific primer we designed here can detect plants infected by SPLV-CY. Their use in combination with the potyviral degenerate primer pair PotI/PotII will enhance the reliability of the approach for diagnosis and detection of SPLV in sweet potato.

For efficient indexing of a large number of samples collected from tissue culture stocks or from field, to develop a specific antiserum with ELISA is necessary. SPLV is difficult to be purified from sweet potato or other hosts for its low titter in the infected tissue. The molecular information elucidated in this study will help us prepare a useful antiserum from the bacteria-expressed CP for large-scale detection of the virus in Taiwan.

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摘 要

王麗媛[1,2]、陳冠君[2]、陳宗祺[2]、葉錫東[2,3]. 2007. 甘藷潛伏病毒中台灣分離株鞘蛋白基因序列分析及於檢測上之應用. 植病會刊 16：141-148. ([1]嘉義市農業試驗所嘉義分所植保系；[2]台中市國立中興大學植物病理學系；[3]聯絡作者，電子郵件：sdyeh@nchu.edu.tw；傳眞：+886-4-22852501)

於嘉義地區，自呈現黃斑及葉脈斑駁的甘藷葉片可分離到似 potyvirus 的長絲狀病毒 (potyvirus-like isolate CY)。利用對應 *Potyvirus* 屬病毒基因體核糖核酸 3' 端之簡併式引子及寡合腺嘌呤引子，可由呈現局部斑之奎藜總量核糖核酸 (total RNA) 以反轉錄聚合酶連鎖反應增幅出約 1.9-kb 核酸片段。此核酸片段經選殖及核酸序列分析，全長含 1880 個核苷酸序列。由 5' 端起此序列對應的胺基酸共561 個，分別爲268 個胺基酸於細胞核內含體 b (nuclear inclusion b, NIb)、及 293 個胺基酸之全長度鞘蛋白 (coat protein, CP) 基因。3' 端非轉譯區 (3' non-coding region, 3'-NCR) 則由 197 個核酸組成。兩者之間蛋白酶切位落於 VHHQ/A 之間，且由鞘蛋白N 端起第 7-9 個胺基酸位置具有被蚜蟲傳播能力之 DAG 序列，不同於 SPLV-TW 之 DTG。與登錄於 GenBank 之SPLV 分離株比對核酸序列，與親緣最近之SPLV-TW 病毒其CP 及 3'-NCR 區域之核苷酸序列相同度分別有 96.5% 及 100%。鞘蛋白基因演化關係分析此病毒和台灣及中國大陸分離株較爲親近而與日本分離株較爲疏遠。根據解得 SPLV-CY 鞘蛋白基因序列，設計之專一性引子對 L166/L841，可由 SPLV-CY 感染之奎藜、煙草及甘藷等病株檢測出專一性 675bp 核酸片段。

關鍵詞：甘藷潛伏病毒、核酸選殖、序列分析、檢測